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Report of the Workshop on Age Reading of Red mullet *Mullus barbatus* and Striped mullet *Mullus surmuletus* (WKACM)

30 March - 3 April 2009

Boulogne sur Mer, France

ICES
International Council for the Exploration of the Sea

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Executive summary

The Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) identified the Red mullet (*Mullus barbatus*) and the Striped mullet (*Mullus surmuletus*) as species requiring an ageing workshop to evaluate and improve the age interpretation based on otolith reading. The Workshop is built on the results of the otolith exchange organized in 2008 and is the first ageing workshop for Red mullet and Striped mullet.

The overall result of the workshop exercises is that there are significant differences in age estimates among readers for the red mullet of the Mediterranean Sea and the striped mullet of the Eastern Channel. The image analysis exercise clarified that the lack of agreement can be due to the interpretation of the first ring for the red mullet of the Mediterranean Sea and the identification of the following rings for both species.

Exploring the application of image analysis, the group agreed that applying such tools in the routine age estimation of the mullets may be very valuable. It gives the opportunity to use metrics to rule out doubt when defining the age structures to count and also gives a very useful exchange tool for the individual readers both within and between laboratories.

The workshop after discussions and analyses identified some problems for the estimated age of the mullets. The group reached agreement on the definition of ageing guidelines mentioned in the present report and the aim is to employ these guidelines to eliminate some of the problems with e.g. false rings in the otolith structures. A collection of agreed aged mullet otoliths were started at the workshop. The reference collection will have to be expanded considerably through others exchange of otoliths and images.

1 Terms of reference

2008/2/ACOM44 A **Workshop on Age reading of Red mullet *Mullus barbatus* and Striped mullet *Mullus surmuletus*** [WKACM] (Chair: Kelig Mahé*, France and Chryssi Mitilineou*, Greece); will be held in Boulogne sur Mer (France), 6–10 April 2009.

a) Review information on age determination, and validation on these species;

b) Compare different otolith-based age determination methods;

c) Identify sources of age determination error in terms of bias and precision: i.e. analyse different validation techniques and describe the corresponding interpretational differences between readers and laboratories, and agree on a common ageing criteria;

d) Analyse growth increment patterns and provide specific guidelines for the interpretation of growth structures in otoliths;

e) Create a reference collection of otoliths and start the development of a data base of otolith images.

The Workshop is an otolith exchange exercise for both *Mullus* species (i.e. *Mullus barbatus* and *Mullus surmuletus*) started during 2007 aimed to deal with possible problems of *Mullus* ageing. Up to now France, Spain, Italy, Cyprus, UK and Greece are participating in the otolith exchange exercise.

WKACM will report by 21 April 2009 for the attention of PGCCDBS and ACOM.

Supporting information

Priority:	Age determination is an essential feature in fish stock assessment to estimate the rates of growth and mortality. In order to arrive at appropriate management advice ageing procedures must be reliable. Otolith processing methods and age reading methods might differ considerably between countries. Therefore, otolith exchanges should be carried out on a regular basis, and if serious problems exist age reading workshops should be organised to solve these problems.
Scientific justification and relation to action plan:	The aim of the workshop is to identify the problems in Mullus spp. age determination, improve the accuracy and precision of age determinations and spread information of the methods and procedures used in different ageing laboratories. A number of samples of otoliths is circulating (2007) among different laboratories to assess the precision of age readers. At the workshop, in 2008, results from the otoliths circulation will be presented and discussed.
Resource requirements:	
Participants:	In view of its relevance to the DCR, the Workshop is expected to attract wide interest from both Mediterranean EU and ICES Member States.
Secretariat facilities:	
Financial:	Additional funding will be required for facilitate the attendance of the scientists. The workshop will be eligible under the E.U. - DCR.
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	
Linkages to other organizations:	There is a direct link with the EU DCR and outcomes from this Workshop will be of interest to several RFOs

2 List of participants and Agenda

The list of participants is presented in Annex 1, and Agenda in Annex 2.

3 Review information on age determination, and validation work on these species (ToR a)

3.1 Review on the biology of *Mullus surmuletus* in the north-east Atlantic

The striped red mullet is found along the European coasts from South Norway (Wheeler, on 1978) and North Scotland (Gordon, on 1981) including Faeroes (Blacker, on 1977), south to the Strait of Gibraltar, and also in the north part of western Africa and in the Mediterranean and Black Seas. It is infrequent off Norway, around Ireland, the north coasts of England and the West of Scotland (Pethon, 1979; Minchin and Molloy, 1980; Davis and Edward, 1988; Gibson and Robb, 1997).

In the Eastern Channel since 1988, the young individuals are distributed in coastal areas, while the adults have a more offshore distribution in the east part (Mahé *et al*., 2005). Finally, nurseries are located in Bay of Saint-Brieuc and Falklands coasts (Morizur *et al*., 1996).

The striped red mullet is a benthic schooling fish. It seems to prefer deep waters and elevated temperatures, and tolerates low and high salinity (corresponding respectively to the habitats of the juvenile and adults) and is rarely found in the transitions zones of intermediate salinity. This species prefer sandy sediments (Mahé *et al*., 2005).

The growth study in the Eastern English Channel and the south of the North Sea (Mahé *et al*., 2005) highlighted a sexual dimorphism expressing a linear and ponderal growth faster for the females compared to the males. The striped red mullet reproduce from May to September with a maximum in June in the North-East Atlantic (Mahé *et al*., 2005) and reaches the size at first sexual maturity at 16.2 cm for the males and 16.9 cm for the females. The period of reproduction is the same in Mediterranean Sea (Lalami, 1971; Hashem, 1973 ; Gharbi & Ktari, 1981).

Mahé *et al*. (2005), analysing the marginal increment, showed that the months of lowest values are February to April (Fig. 1) and therefore the possible period of annulus formation is winter to spring. Similar results were found by Reňones *et al*. (1995) analysing the percentage of individuals with opaque edge (Fig. 2).

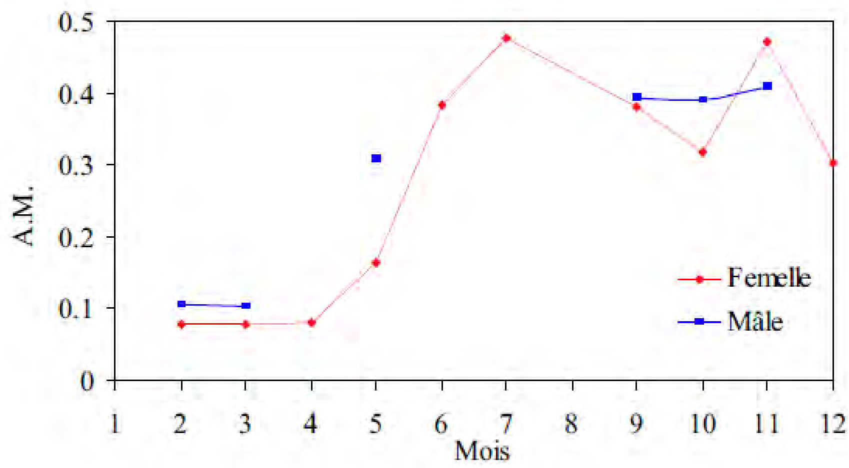

Figure 1 : Marginal-increment (AM) per month (mois) (*In* Mahé *et al*., 2005).

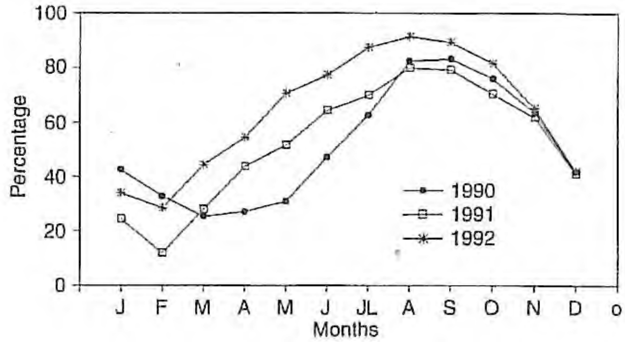

Figure 2 : Monthly variations in percentage of individuals with opaque otolith marginal rings in1990 (n=1220), 1991(n=970), 1992 (n=1095) (*In* Renones *et al.*, 1995).

3.2 Review on the biology of *Mullus barbatus* in the Mediterranean Sea

The red mullet, *Mullus barbatus*, is distributed in the eastern Atlantic, along the European and African coasts from the North Sea and England to Senegal and also in the Mediterranean and Black Seas (Fisher *et al.*, 1987, Tserpes *et al.*, 2002 and references therein). It is very common in the Mediterranean but quite uncommon in the Atlantic (Bauchot & Pras, 1980). The species has gregarious behaviour.

In the Mediterranean, the red mullet is frequently found on muddy bottoms at depths ranging between 5 and 250 m (Tserpes *et al.*, 2002 and references therein). In summer, juveniles are concentred very close to shore, while in autumn, they move towards deeper bottoms (Vassilopoulou & Papaconstantinou, 1987; Voliani, 1999).

According to Voliani (1999), the maximum total length (TL) of red mullet in the Mediterranean is 28-29 cm in females and 23 cm in males.

Red mullet reproduction takes place near the coast; in some areas from May to June-July (Gharbi & Ktari, 1981; Cherif *et al.*, in press), whereas in others from April to August (Vassilopoulou & Papaconstantinou, 1991). The length at first maturity is around 11-12 cm TL for both males and females (Vassilopoulou & Papaconstantinou, 1991)

Eggs, larvae and post larvae up to 30-35 mm of *M. barbatus* are pelagic and live in surface waters. According to Sabatés & Palomera (1987), in the Mediterranean Sea, larvae are found strictly in surface waters (0-1.5 m depth) mainly between June and July.

Juveniles up to 4-5 cm TL are pelagic; beyond this size, juveniles become demersal. Recruitment occurs in coastal bottoms in summer–early autumn (Vassilopoulou & Papaconstantinou, 1991; Levi *et al.*, 2003; Kalagia *et al.*, 2004) at lengths 5-6 cm (Voliani, 1999).

Several published works exist on age and growth of the species in the Mediterranean Sea (Gharbi & Ktari, 1981; Vassilopoulou & Papaconstantinou, 1991; Fiorentino *et al.*, 1998; Kalagia *et al.*, 2004 ; Tsamis *et al.*, 2006) , however, no agreement is always obvious among their results. The maximum estimated age in years among the exploited mediterranean stocks is 7 years for females and 6 years for males (Vassilopoulou & Papaconstantinou, 1991); However, 5 age groups for females and 4 for males is reported in most of studies (Gharbi & Ktari, 1981; Livadas, 1989; Tursi *et al.*, 1994).

Marginal increment analysis for the species in the Greek waters indicated that annuli are formed from spring to summer (Kalagia *et al.*, 2004).

4 Compare different otolith-based age determination methods (ToR b)

For the striped red mullet, 2 methods were used for the age reading during the otolith exchange:

- Reading of digital images of Whole otoliths without any treatment
- Reading of digital images of Burnt Whole otoliths (burning had been performed on the concave face of the otolith in 150°C during few seconds)

Figure 3: Whole otolith (a) and Burnt Whole otolith (b).

4.1 New analysis

The first analysis of the relults of the exhange exercise, including all the readers for each set of otoliths (14 readers for the set of whole and 10 readers for the set of burnt whole otoliths) revealed differences between the results of the two methods (see 5.3.1: results of the exchange). However, during the workshop, a new analysis was applied between the results of the same readers (the 10 common readres) in order to compare the two methods.

The results derived by the new analysis of the exchange sets of burnt and unburnt otoliths showed that burnt otoliths of *M. surmuletus* presented the same agreement and CV values than the unburnt ones for the same set of the images (63 images) and the same readers (10 readers).

4.2 Whole otoliths

The total analysis for the entire otoliths for all readers combined revealed an overal average agreement of 72.6% (ranging between 38-92% among the individual otoliths) and a CV of 26.3% (ranging between 11-361% among the individual otoliths).

4.3 Burnt Whole Otoliths

The total analysis for the burnt otoliths for all readers combined revealed an overal average agreement of 71.6% (ranging between 40-100% among the individual otoliths) and a CV of 25.7% (ranging between 0-316% among the individual otoliths).

5 Identify sources of age determination error in terms of bias and precision (ToR c)

5.1 Summary of the exchange prior to WKACM

5.1.1 Introduction

In March 2005, the Planning Group on Commercial Catch, Discards and Biological Sampling (PGCCDBS) agreed about the necessity of organizing an otolith exchange exercise for both *Mullus* species (i.e. *Mullus barbatus* and *Mullus surmuletus*) aiming to deal with problems of these species ageing.

The planning group had decided Greece, represented by the Hellenic Centre for Marine Research (HCMR), to be in charge of the otolith exchange exercise for the *Mullus* species, and to organize it in 2006. All the relevant European Institutes were contacted and finally 6 countries (Greece, Cyprus, France, Italy, Spain & UK) and 11 Institutions (including 29 persons) expressed their interest in participating in the exchange.

The objectives of the exchange were 1) to investigate the level of agreement on age reading, 2) to analyse the relative differences between the participants and 3) to define the necessity of a workshop on *Mullus* species ageing.

5.1.2 Methods

The first step was the exchange of information. In this framework, a questionnaire was prepared by HCMR in order to collect and exchange information concerning the material and methodology used and the experience of each Institute on Mullus ageing.

After a detailed discussion, the group decided to follow the EFAN report 3-2000 guidelines concerning the age otolith exchange exercises. Two sets of otoliths were chosen, one (60 otoliths) from the CNR-IAMC collection for M. barbatus of the Sicily Channel and another (63 otoliths) from the IFREMER collection for M. surmuletus of the English Channel. The selected set of *M. surmuletus* otoliths (IFREMER) included also burnt otoliths, which were also examined by some of the readers. Digital images of the selected otoliths were used for the age reading. In order to maintain the same methodology and to collect the same information, HCMR prepared a protocol for the readers to follow. HCMR developed also a web -page to give access to the digital images and the results of the age reading.

The data of each species were analysed for all readers combined as well as for each reader separately. The overall work lasted from September 2006 to June 2007.

5.1.3 Results

5.1.3.1 Mullus barbatus

The total analysis for all readers combined revealed an overal average agreement of 51.6% (ranging between 29-86% among the individual otoliths) and a CV of 68.5%.

The CV, percent agreement and standard deviation by modal age are presented in Fig. 4. The CV values were decreasing with age.

The high CV values of modal age 0 indicated serious problems in 0 group ageing. The examination of the relative bias indicated an overestimation of the young ages and a subestimation of the older ones.

The coefficient of variation, the percent agreement and the relative bias by age reader are presented in Table 1. Mediterranean age readers were more efficient than the northern European counterparts.

Table 1. The coefficient of variation (CV), the percent agreement and the relative bias for each participant age readings of *M. barbatus* otoliths.

	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13	Reader 14
CV%	22,80%	33,40%	47,00%	37,80%	43,50%	37,10%	73,50%	76,20%	34,40%	21,00%	41,20%	96,90%	83,40%	51,40%
Agreement %	78,30%	69,50%	86,70%	63,30%	64,90%	60,00%	55,00%	55,20%	29,30%	13,60%	35,00%	45,00%	38,30%	28,30%
Relative Bias	0,05	-0,31	0,08	-0,1	-0,23	0,43	0,4	-0,52	0,84	1,46	0,87	-0,62	-0,67	0,82

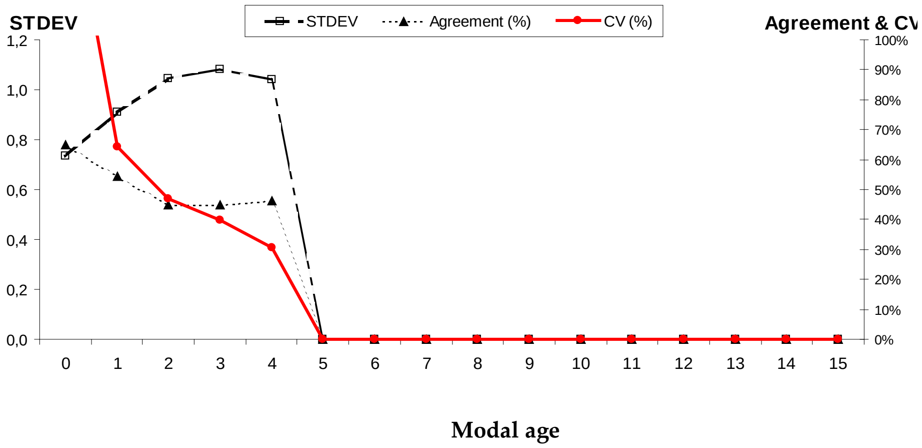

Figure 4: The coefficient of variation (CV%), the percent agreement and the standard deviation (STDEV), from all readers combined, plotted against the MODAL age of the *M. barbatus* otoliths age readings.

5.1.3.2 Mullus surmuletus

5.1.3.2.1 Whole otoliths

The total analysis for the whole otoliths for all readers combined revealed an overal average agreement of 64.3% (ranging between 38-92% among the individual otoliths) and a CV of 60.7%.

The CV, percent agreement and standard deviation by modal age are presented in Fig. 5. The CV values were decreasing with age. The high CV values of modal age 0 indicated serious problems in 0 group ageing. The examination of the relative bias indicated an overestimation of the young ages and a subestimation of the older ones.

The coefficient of variation, the percent agreement and the relative bias by age reader are presented in Table 2. Northern European age readers were more efficient in age reading than the Mediterranean counterparts.

Table 2. The coefficient of variation (CV), the percent agreement and the relative bias for each participant age readings of *M. surmuletus* whole otoliths.

	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10	Reader 11	Reader 12	Reader 13
CV%	3,60%	11,40%	9,10%	13,10%	16,00%	11,30%	22,20%	44,20%	12,30%	21,00%	62,80%	19,20%	38,70%
Agreem.%	95,20%	87,30%	87,30%	79,00%	73,00%	58,10%	37,10%	79,40%	52,40%	57,40%	49,10%	30,60%	44,30%
Rel. Bias	0,05	-0,08	0,13	-0,08	-0,16	-0,42	-0,03	0,21	-0,63	-0,61	0,26	0,47	0,62

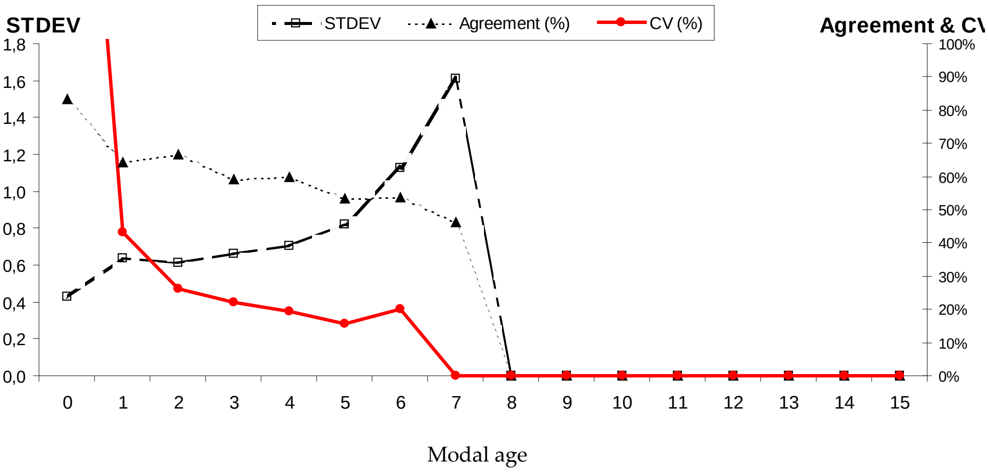

Figure 5: The coefficient of variation (CV%), the percent agreement and the standard deviation (STDEV), from all readers combined, plotted against the MODAL age of the *M. surmuletus* whole otoliths age readings.

5.1.3.2.2 Burnt Whole otoliths

The total analysis for the burnt whole otoliths for all readers combined revealed an overal average agreement of 71.6% ranging between 40-100% among the individual otoliths and a CV of 25.7%.

The CV, percent agreement and standard deviation by modal age are presented in Fig. 6. The CV was slightly decreasing with age. The relatively high CV of modal age 0 indicated problems in 0 group ageing. The examination of the relative bias indicated an overestimation of the young ages and a subestimation of the older ones.

The coefficient of variation, the percent agreement and the relative bias by age reader are presented in Table 3. Northern European age readers were more efficient in age reading than the Mediterranean counterparts.

Table 3. The coefficient of variation (CV), the percent agreement and the relative bias for each participant age readings of *M. surmuletus* burnt otoliths.

	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	Reader 10
CV%	9,60%	10,00%	14,70%	14,90%	17,20%	13,50%	13,10%	50,90%	20,80%	48,70%
Agreem.%	87,30%	88,90%	73,00%	84,10%	71,40%	60,00%	65,10%	71,00%	56,70%	51,00%
Rel. Bias	-0,03	0,06	-0,11	0,14	-0,13	-0,32	-0,44	0,24	-0,38	0,37

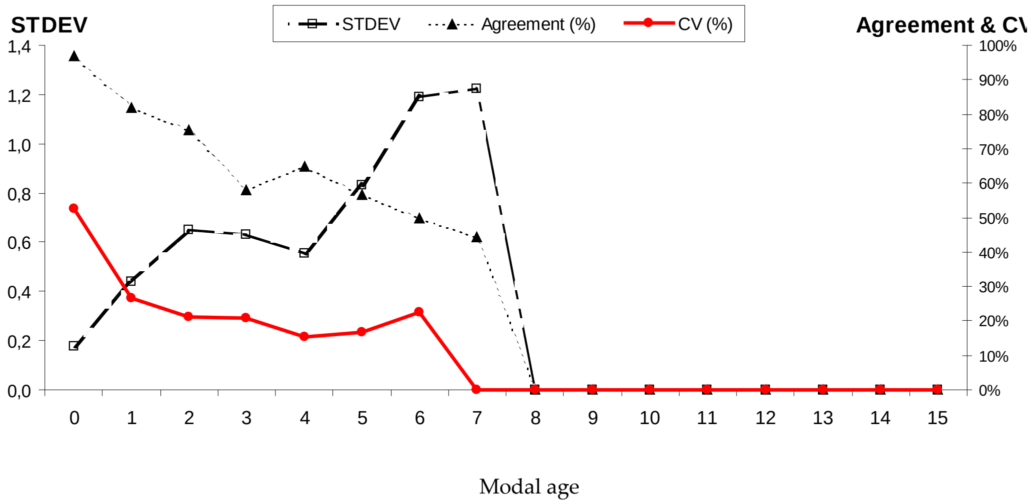

Figure 6: The coefficient of variation (CV%), the percent agreement and the standard deviation (STDEV), from all readers combined, plotted against the MODAL age of the *M. surmuletus* burnt whole otoliths age readings.

5.1.4 Conclusions

The results of *M. barbatus* and *M. surmuletus* otolith exchage exercise indicated that ageing of both species could not be considered as easy. The agreement was in all cases low and the CV was high, particularly for the mediterranean set of *M. barbatus* otoliths. Although the main objectives of the exchange exercise was the identification of differences between readers and the necessity of a workshop, the following remarks could also be made:

Better results were obtained for *M. surmuletus* than *M. barbatus*. This could be related with the clearer appearance of rings in the otoliths of the former species.

Mediterranean age readers gave generally better results for the mediterranean set of *M. barbatus* otoliths (fact that could be related with their experience on the mediterranean growth pattern) compared with their north European colleagues. The opposite occured with the north European set of *M. surmuletus* otoliths.

5.2 Sources of bias

5.2.1 *M. surmuletus*

From the discussion during the workshop the following remarks were pointed out:

- Disagreement in the identification of the first annual ring; one group of the readers considered the first ring as the ring of settlement, whereas the majority considered it as the first annual ring.

- Disagreement in the identification of other rings.
- Confusion concerning the protocol of reading during the exchange; some of the readers considered as date of birth the 1st of January, whereas others considered as date of birth the 1st of June.
- Confusion concerning the axis of the otolith used for the measurements.

5.2.2 *M. barbatus*

The discussion during the workshop revealed the following remarks:

- Low quality of digital images;many otoliths were very transparent and consequently of low clearness.
- Disagreement in the identification of the first annual ring; there was a great variability in what was considered as first annual ring. As a result the radius of the first annulus presented a great variability between the readers. Some of the readers considered the first very clear ring as the ring of settlement.
- Disagreement in the identification of other rings.
- Confusion concerning the protocol of reading during the exchange; some of the readers considered as date of birth the 1st of January, whereas others considerd as date of birth the 1st of June.

5.3 Analysis of various validation techniques

According to the results of the exchange, one of the serious problems in the age reading of both *Mullus* species was the high values of CV for the first annual ring. Therefore, the participants of the workshop, after a thorough discussion, decided to focus their effort to the identification of the first annulus. In order to clarify the discrepancy problems between the readers, the following procedures were applied:

- application of statistical tests to compare the results of the readers in total and per age group according to Eltink *et al.* (2000) protocol
- graphical representation of the measurments on the radius of the first annulus per each reader
- back-calculation of lengths for the first annulus
- use of information concerning the different cohorts and the new recruits from the length frequency composition of the stock whose otoliths were examined
- use of available information concerning the marginal increament from the bibliography

However, in order to have comparable and more objective results, the above procedures as well as the back-calculation of lengths for each annulus and comparisons of them, were repeated again for the results of the blind rereading experiment. More validation techniques (e.g. daily increament analysis, tagging, marginal increament analysis, etc) should be applied in the future.

5.4 Agreement on a common ageing criteria

The following procedures were agreed as a common protol for the age reading.

As a first step:

- a blind re-reading of the otoliths without any information related with the otoliths (e.g. length) except on the date of capture of the specimens.
- consider the 1st of January as date of birth for both species (Mediterranean partners suggested that 1st of June is a more appropriate date for both *Mullus* Mediterranean species; however in order to follow the general guidelines considered by other ageing workshops, they accepted it as a rule for this agreement)
- hyaline ring at the edge of the otolith during the first semester of the year is considered as annulus.
- hyaline ring at the edge of the otolith during the second semester of the year is not considered as annulus.
- Measurements should be done on the axis derived between the sulcus and the *nucleus*.

Proceed to a draft check of the identified rings according to the measuments of their radius. Then continue with a new reading based on the following procedures:

- comparison of the differences in the increament between the consecutive annuli

6 Analyse growth increment patterns and provide specific guidelines for the interpretation of growth structures in otoliths (ToR d)

6.1 Definition of annuli and date of birth

Otolith (*sagittae*) of *M. barbatus* and *M. surmuletus* display annual growth increments, called annuli. Each annulus consists of one opaque and one translucent zone. The opaque zone represents the period of fast growth (summer ring) and the translucent zone represents the period of low growth (winter ring). In both species, a complete annulus was visible in summer, when an opaque zone of the new year has started to be formed outside the translucent zone of the previous year. This transition could sometimes start to be observed in late spring (May).

Date of birth is considered to be the 1st of January. This implies that translucent zone at the edge of otolith during the first part of the year shall be interpreted as the translucent zone that follows the previous year opaque zone, and should be counted as one annulus.

Mediterranean partners suggested that maybe 1st of June is more appropriate date of birth for both *Mullus* mediterranean stocks, since this is more close to the pick of the reproduction period in the area; however in order to follow the general guidelines considered also by other ageing workshops, they accepted it as a rule for agreement purposes, considering that the formation of annuli occures a little before the real biological age of the species.

6.2 Rereading experiment and Interpretation of the first *annullus*

During the workshop and in order to evaluate if the discussions have made an improvement in the age reading, a second blind reading was carried out following the new agreement on the common ageing criteria, which was decided by the participants of the WK. Forty images were selected for *M. barbatus* and thirty for *M. surmuletus*. All the images were analysed using the software TNPC. Each reader had the posssibility to annotate the false and the real rings by different marks without any idea of their distances from nucleus, although these measurements were saved into a separate file. Back-calculation was applied to compare interpretations between the various readers for the various rings and particularly the first one. Eltink *et al.* (2000) spreadsheet was also used to compare the agreement and precision between the readers in total and per age group after the blind rereading experiment.

6.2.1 M. *barbatus*

In this species, an observable ring was identified by most readers before the first annual ring and considered as false (Fig. 7; Annex 3). However, one of the readers considered all identified rings as real annuli. Moreover, there was confusion between the readers concerning the position of the false ring and the position of the first annual ring. Back-calculation of the length of the fish of each image, based on the false and the first annual rings defined by the six readers, presented a great variability for both rings (Fig. 8 & 9; Annex 3). Furthermore, the lengths of the false and first annual rings showed an important overlapping among some of the readers. It seemed that this was not the case for the second annulus (Fig. 10).

Statistical analysis for the median of the 1st annulus revealed significant differences between the readers (Kruskal-Wallis, $p<0.05$). Two homogeneous groups were statistically found (Gr1: readers 2, 4 & 6; Gr2: readers 3 & 5), whereas reader 1 differed significantly from all other readers. Statistical analysis for the median of the 2nd annulus showed also statistical differences among the readers ($p<0.05$). Three homogeneous group could be detected (Gr1: readers 2, 4, 5 & 6; Gr2: readers 2, 3, 4 & 5; Gr3: readers 1 & 6). The mean, the median, the 70% of the values, the min and max and the outliers for the back calculated lengths of the 1st and 2nd annulus for each reader are represented by the box and whisker plots in Annex 4.

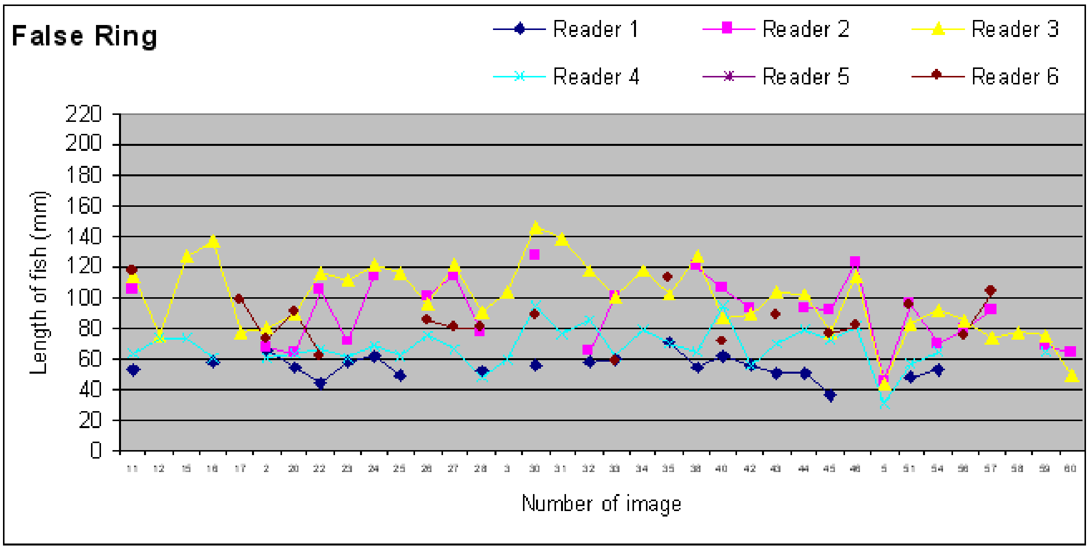

Figure 7: ***M. barbatus:*** **Back-calculation of the fish length (mm) for the position of the false growth ring for each image and each reader.**

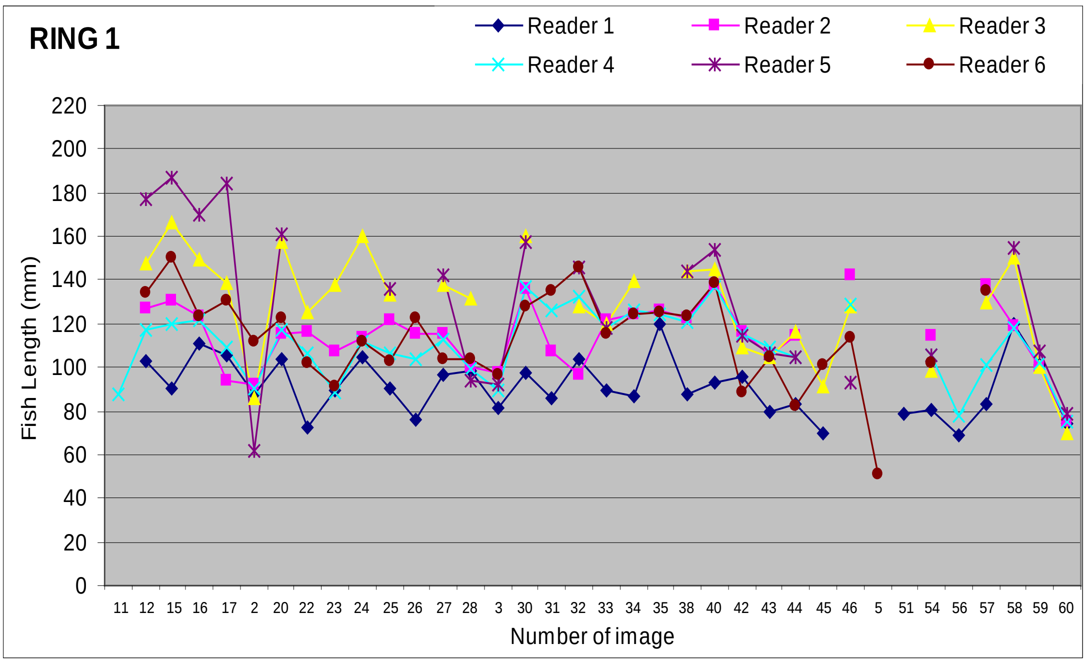

Figure 8: ***M. barbatus:*** **Back-calculation of the fish length (mm) for the position of the first growth ring for each image and each reader.**

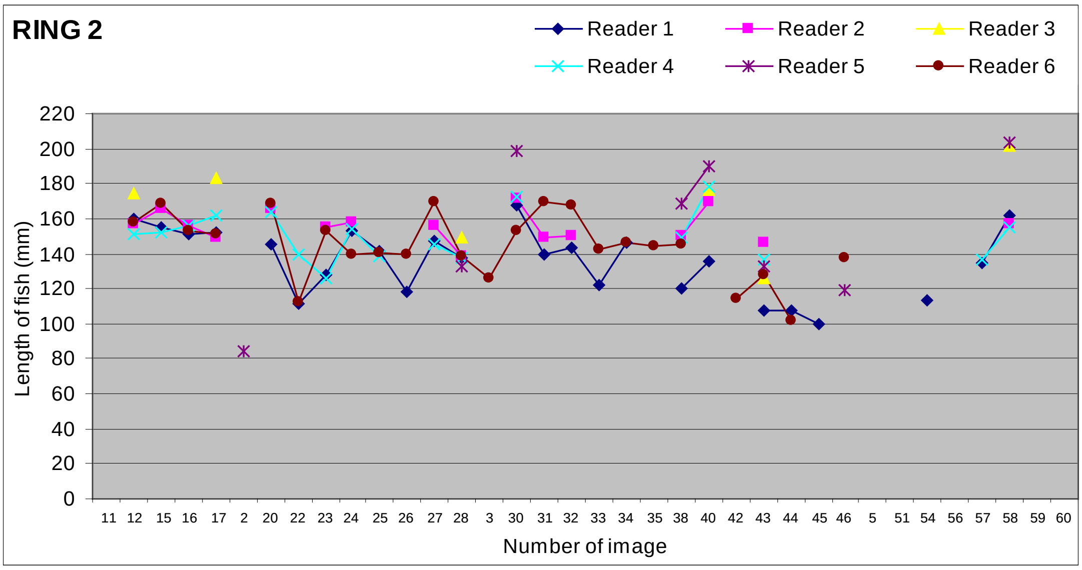

Figure 9: ***M. barbatus:*** **Back-calculation of the fish length (mm) for the position of the second growth ring for each image and each reader.**

There is confusion between the readers to position the first ring of growth with the false ring. There isn't this problem for the second ring of growth (Fig. 10).

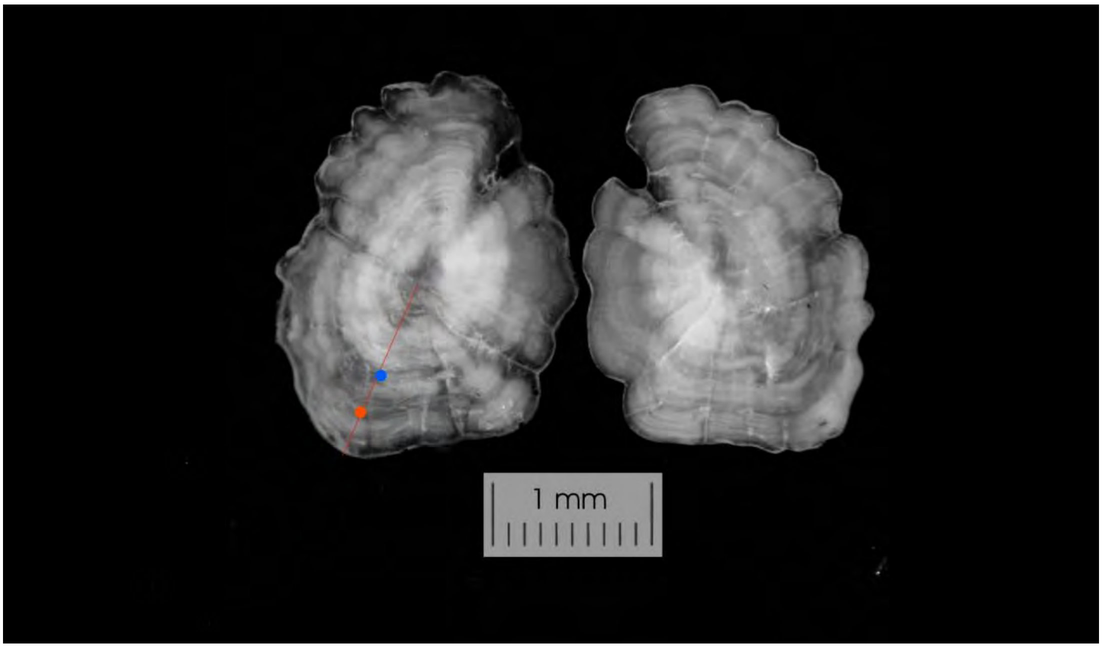

Figure 10: Image of ***M. barbatus*** **(In blue: the first growth ring or a false ring; in orange: the growth ring for all readers).**

The diameter of the first annulus is expected to vary among individual specimens following the variability in growth related to the range of the reproductive period. *M. barbatus* in the Mediterranean exhibits a reproductive period ranging from April to August. Thus, a variability of the first annulus radius is expected as well as a variability of the corresponding somatic length. According to the literature (see 3.2), a) juveniles are pelagic up to 40-50 mm and afterwards become demersal and b) the new recruits appear mainly in late summer-autumn at lengths more than 60 mm.

Consequently, we could suppose that the ring appearing at around 60-80 mm of somatic length (as identified by some of the readers) could be a false ring because of the transition from the pelagic to the demersal life. This hypothesis could be supported by the structure of the length composition of the studied stock, examined during the workshop, which showed a clear mode of new recruits at 60-70 mm of length and a second one at around 120-130 mm in Autumn as well as by our knowledge on a spring formation of the annuli (see 3.2 ; Fig. 11).

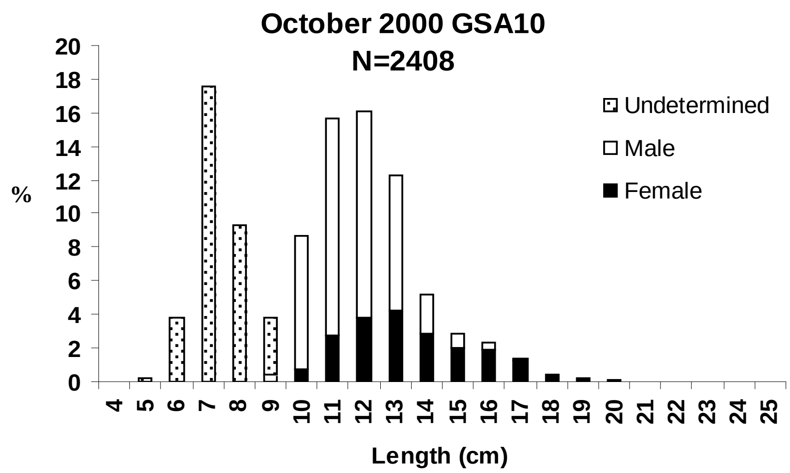

Figure 11: Length frequencies of *M. barbatus* from an autumnal trawl survey.

The maximum identified number of annuli for the studied set of images was 6. The number of identified annuli differed among the readers for most images. As a result, the total analysis of the rereading 36 images using the Eltink *et al.* (2000) spreadsheet revealed an overal average agreement of 57.9% (ranging between 33-100% among the individual otoliths) and a CV of 71.9% (ranging between 0-245%) (Fig. 12). In comparison with the same set of images and readers, the analysis of the results of the exchange experiment revealed for the first reading an overal average agreement of 59.4% and a CV of 65.8%.

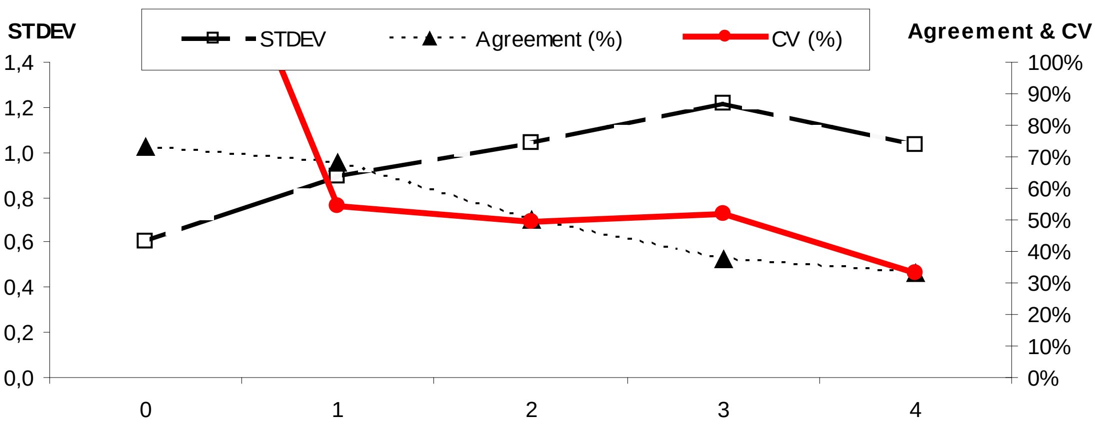

Figure 12: The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) for the *Mullus barbatus* are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

More validation techniques are necessary, since the interpretation of the first annulus was not obvious. Moreover, taking into consideration the low quality of the studied images of *M. barbatus*, a new set of otoliths is proposed to be read in the future.

6.2.2 M. *surmuletus*

In this species, only two of the readers identified in most images a false ring before the first annulus, whereas the others only sporadically (Fig. 13). The back calculated lengths of this false ring were around 100 mm and always lower than 155 mm TL.

The first annual ring identified in all images and by all readers showed a considerable coincidence in its measure among all readers as well as its back-calculated lengths, which ranged mainly between 160-190 mm TL (Fig. 14) with an average at 175 mm.

Other false rings were identified between the following annuli mainly by three of the readers presenting more or less the same value each one among the readers. The back-calculated lengths for the second false ring ranged mainly between 200-250 mm TL (Fig. 13). False rings were less obvious with age.

The second annulus showed also a considerable coincidence among the readers and its back-calculated lengths ranged mainly between 230- 280 mm TL (Fig. 15) with an average at 255 mm. This was also the case for the 3rd (Fig.16), which ranged mainly between 260-310mm with an average at 288mm. The fourth ring ranged between 300-340mm with an average at 313 mm TL.

Although statistical analysis for the median of the 1^{st} annulus revealed significant difference between the readers (Kruskal-Wallis, $p<0.05$), that was related to reader 4; all other readers consisted a homogeneous group. Statistical analysis for the median of the 2^{nd} annulus showed a high homogeneity among all readers ($p>0.05$). The mean, the median, the 70% of the values, the min and max and the outliers for the back calculated lengths of the 1^{st} and 2^{nd} annulus for each reader are represented by the box and whisker plots in Annex 5.

Rings on the otoliths of *M. surmuletus* from the English Channel were generally easily detected. The high coincidence of the annuli measures could be related to the good quality of the images examined as well as to the environmental characteristics (e.g. higher seasonal differences in the temperature) generally in the otoliths of Atlantic stocks results in the formation of more clear and distinguishable annuli (Townsed *et al*., 1992) than their Mediterranean counterparts. Nevertheless, this could consist a term of reference of a next workshop.

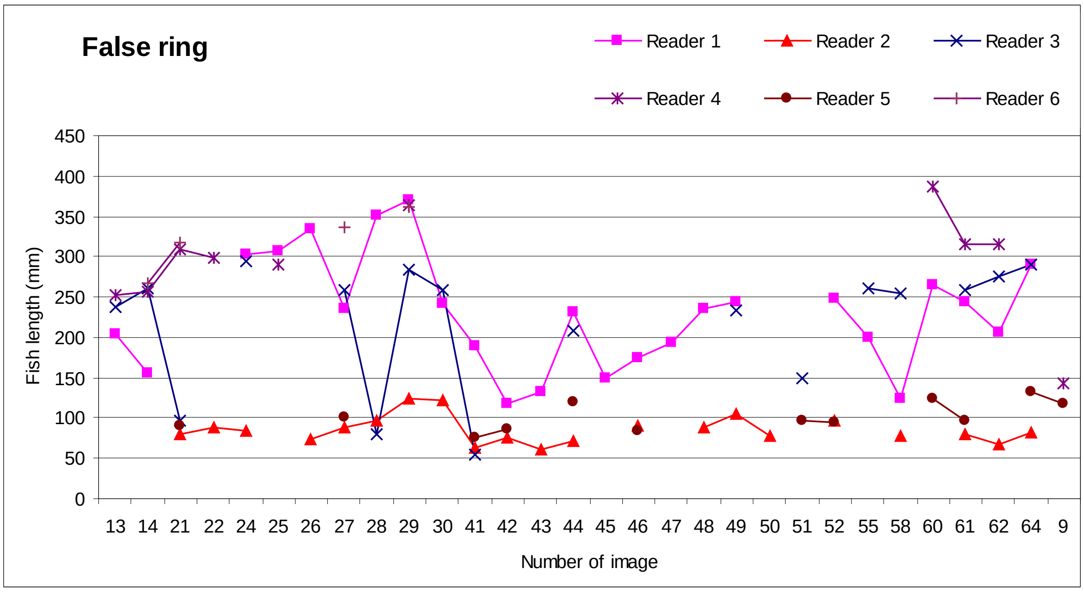

Figure 13: ***M. surmuletus*** **: Back-calculation of the fish length (mm) for the position of the false ring for each image and each reader.**

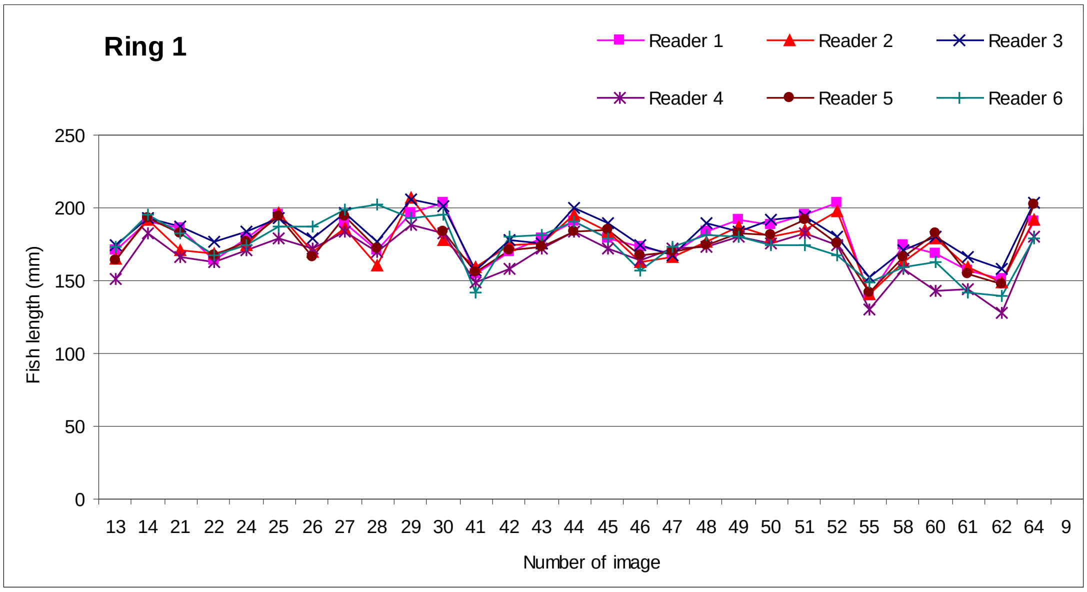

Figure 14 : ***M. surmuletus*** **: Back-calculation of the fish length (mm) for the position of the 1st growth ring for each image and each reader.**

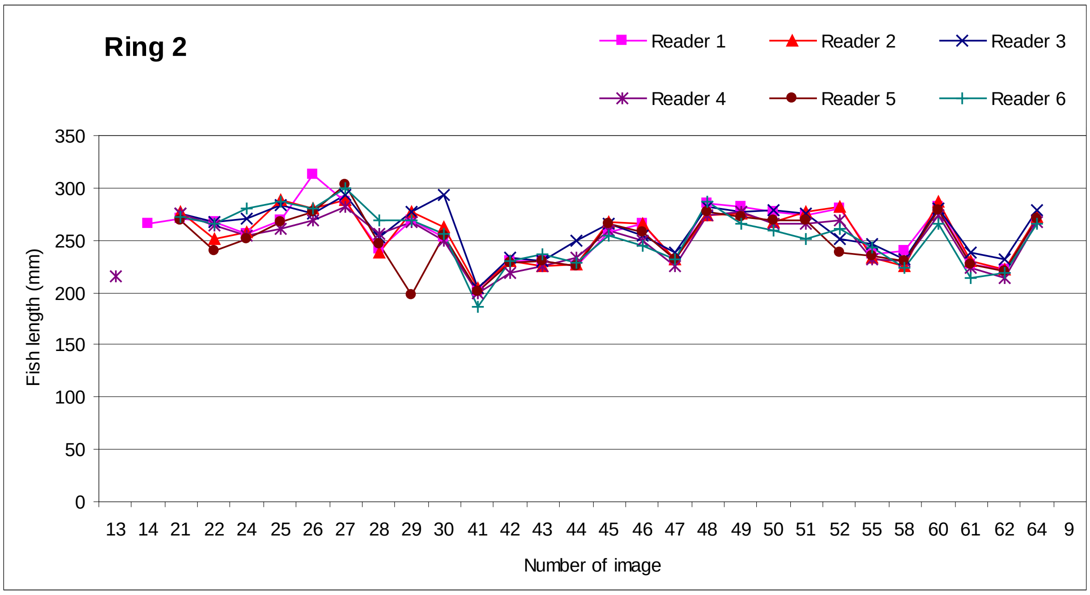

Figure 15 : *M. surmuletus* : Back-calculation of the fish length (mm) for the position of the 2nd growth ring for each image and each reader.

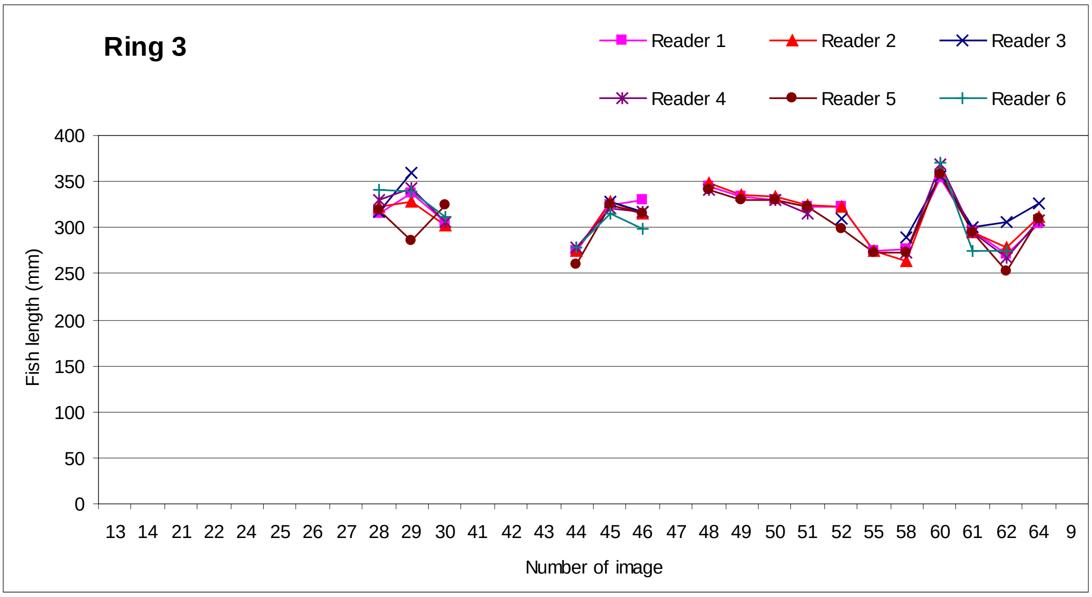

Figure 16. *M. surmuletus* : Back-calculation of the fish length (mm) for the position of the 3rd growth ring for each image and each reader.

The maximum identified number of annuli for the studied set of images was 8. Although a high coincidence was detected among readers for the position of each annulus in this species, the number of identified annuli differed considerably among the readers for most images. As a result, The total analysis for the burnt whole otoliths (30 images) for all readers (6 readers) combined using the Eltink *et al.* (2000) spreadsheet revealed for the rereading experiment an overal average agreement of 64.4% ranging between 33-100% among the individual otoliths and a CV of 19.6% (ranging between 0-33% among the individual otoliths) (Fig. 17). In comparison, the analysis for the exchange reading for the same set of images and readers, revealed an overal agreement of 55.6% and a CV of 29.0%.

Consequently, the rereading experiment provided better results in the age reading of *M. surmuletus*, although these need to be improved in the future. Higher precision is expected, and the use of various validation methods could help this aim.

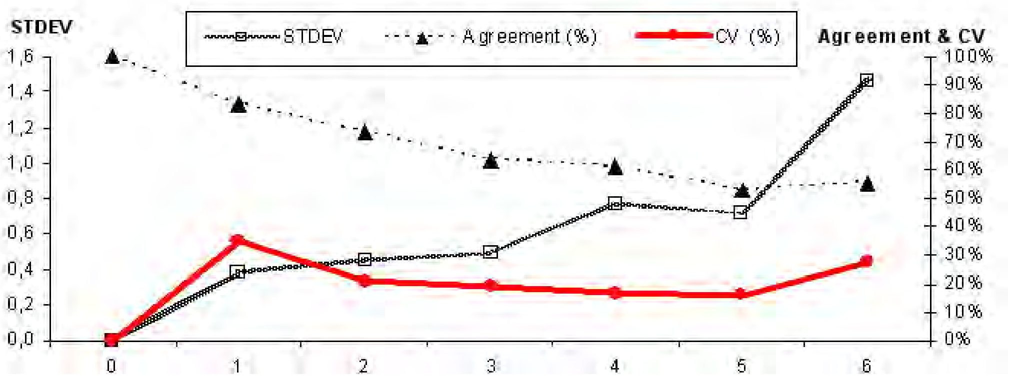

Figure 17. The coefficient of variation (CV%), percent agreement and the standard deviation (STDEV) for the *Mullus surmuletus* are plotted against MODAL age. CV is much less age dependent than the standard deviation (STDEV) and the percent agreement. CV is therefore a better index for the precision in age reading. Problems in age reading are indicated by relatively high CV's at age.

6.3 Guidelines for interpretation of age

After the second reading experiment and the discussion of its results, the readers agreed the following procedures for the age reading and the determination of age:

<u>Otolith selection</u>

- The left otolith shall be used for the age reading as a rule. However, the right otolith could also be useful, in case that the left otolith presents a problem. When the rigth otolith is considered, then the number of annuli shall be taken into account without any measurements.
- If various methods are used (buring, staining, sectionning etc), digital images of the otolith shall be taken before.

<u>First Reading</u>

- As a first step, a blind reading shall be performed at the beginning without any information except the date of capture.
- Selection of a suitable measurement axis; it is proposed the axis joining the *sulcus* and the *nucleus* of the otolith (Fig. 18). Measurements shall be performed on this axis from nucleus to the posterior area of the otolith. This axis must be constant for all the measurements.
- Annotate all the considered false or true rings and measure them, if possible without any idea of these measurements (it depends of the used software).
- Translucent true rings should be visible more o less around the whole otolith in order to be considered as annual rings.
- 1st of January is considered as the date of birth. As a result, if a translucent ring is observed at the edge of the otolith at the first part of the year, then it shall be counted as annulus. In contrary, if a translucent ring is observed at the edge of the otolith at the second part of the year, then it should not be counted as annulus.

- Evaluation of the results of the first blind reading, examination of the variability in the presence of annuli and in their measurments.
- Use various validation methods such as back-calculation, marginal increament analysis, examination of the growth increament between the consecutive rings.
- Evaluate all these results taking into consideration available biological information concerning the species such as, the period of presence of new recruits and their lengths, the modes in the length composition of the stock, the period of annulus formation.

Second Reading

- Proceed to a new reading of the otoliths taking into consideration the following:
  - all available information for the otolith (date of capture, length of specimen, sex, maturity stage etc)
  - all available biological information for the species
  - the results of the first reading
  - the radius of each annulus and the result of a draft back-calculation of the length of the species the time of the annulus formation according to the formula: $L_n = L_t * (R_n/R)$, where L_n is the length of the specimen at the ring R_n, R is the radius of the otolith and L_t is the actual length.
  - the increament ($R_{n+1} - R_n < R_n - R_{n-1}$) between the consecutive annuli should be decreasing with age

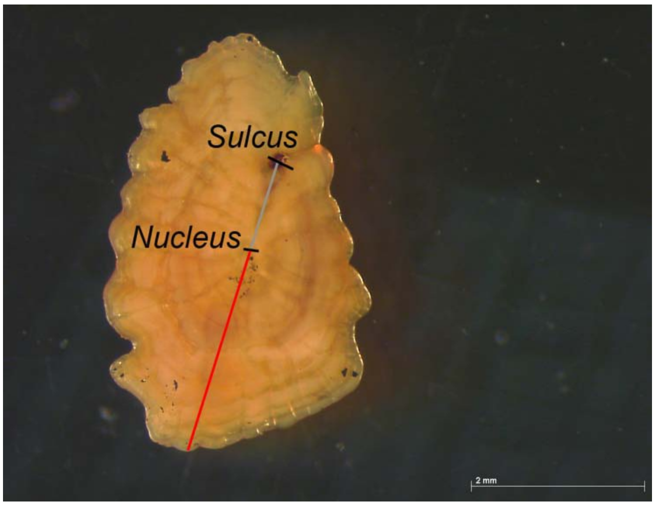

Figure 18: The suitable measurement axis from the *sulcus* to the *nucleus* and measure from *nucleus* to the posterior area of otolith (red line).

7 Create a reference collection of otoliths and start the development of a data base of otolith images (ToR e)

Since agreement in the age reading for both *Mullus* species was low among the readers, few images were selected as good examples to start create a reference collection. The following images were chosen by all the readers:

7.1 *M. surmuletus*

Figure 19 : Image of *M. surmuletus* (age : 1 ; length : 280mm , Sex : M , month of capture : 10).

Figure 20 : Image of *M. surmuletus* (age : 3 ; length : 340mm , Sex : F , month of capture : 10).

Figure 21 : Image of *M. surmuletus* (age : 2 ; length : 350mm , Sex : F , month of capture : 10).

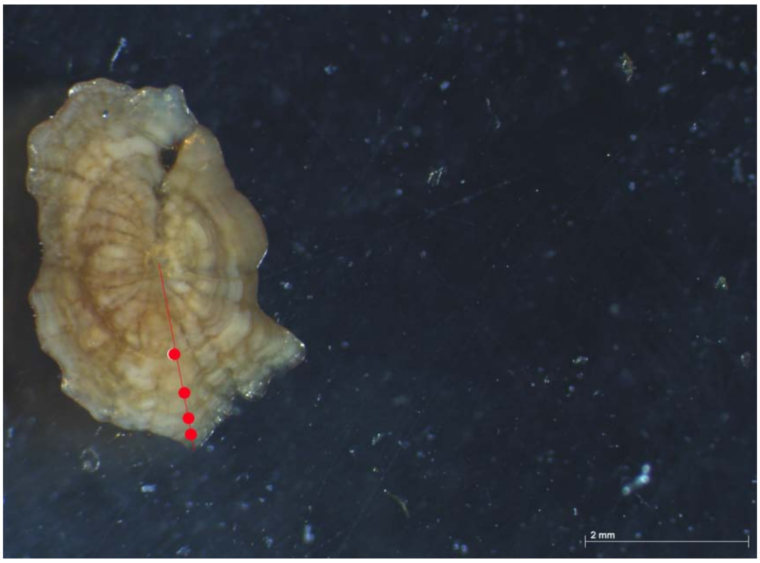

Figure 22 : Image of *M. surmuletus* (age : 4 ; length : 360mm , Sex : F , month of capture : 10).

7.2 *M. barbatus*

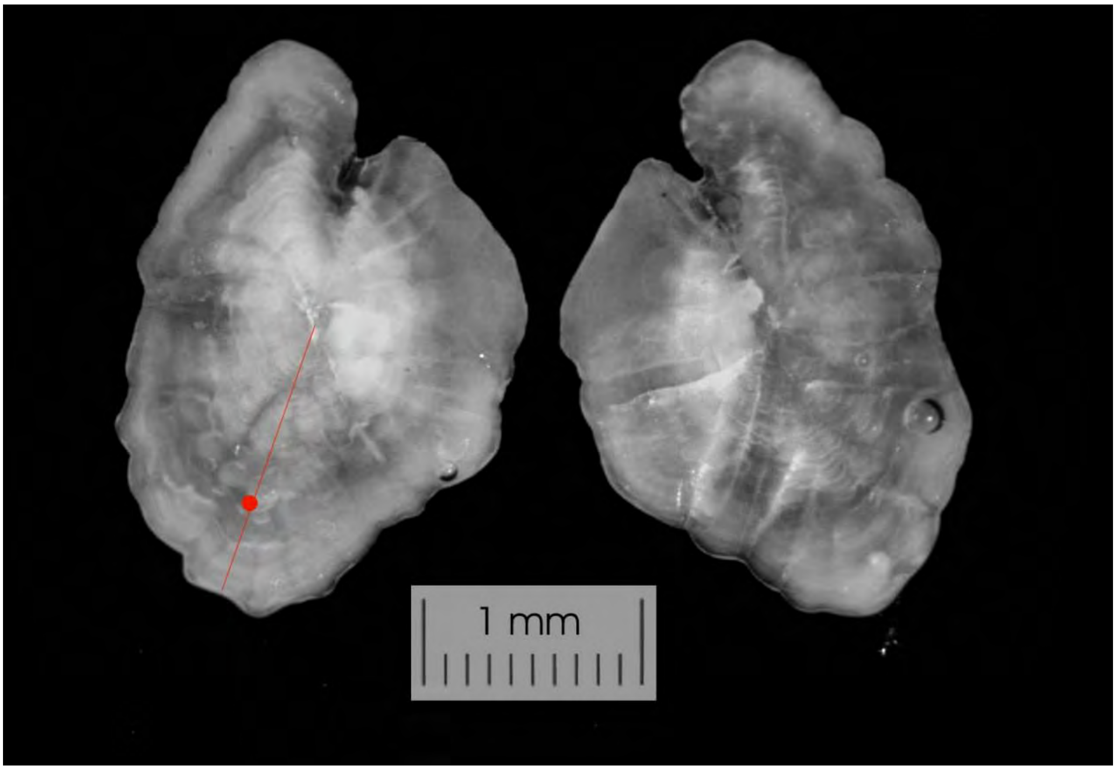

Figure 23 : Image of *M. barbatus* (age : 1 ; length : 130mm , Sex : M , month of capture : 7).

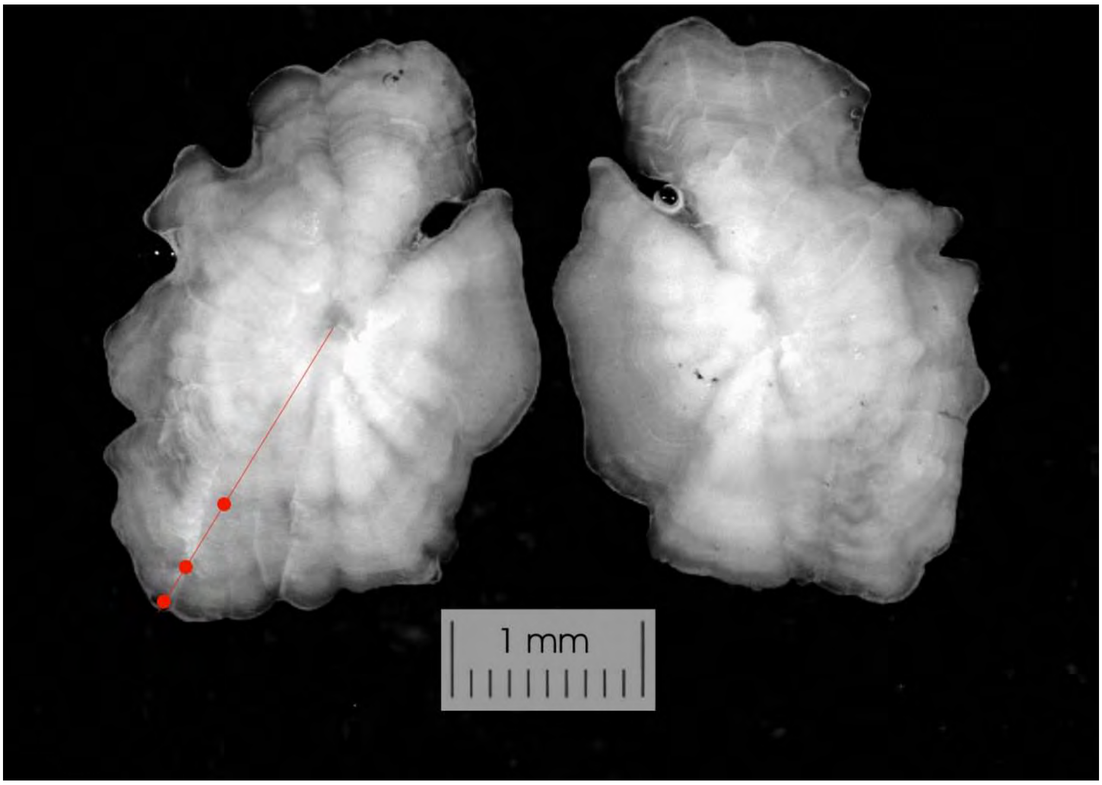

Figure 24 : Image of *M. barbatus* (age : 3 ; length : 160mm , Sex : M , month of capture : 6).

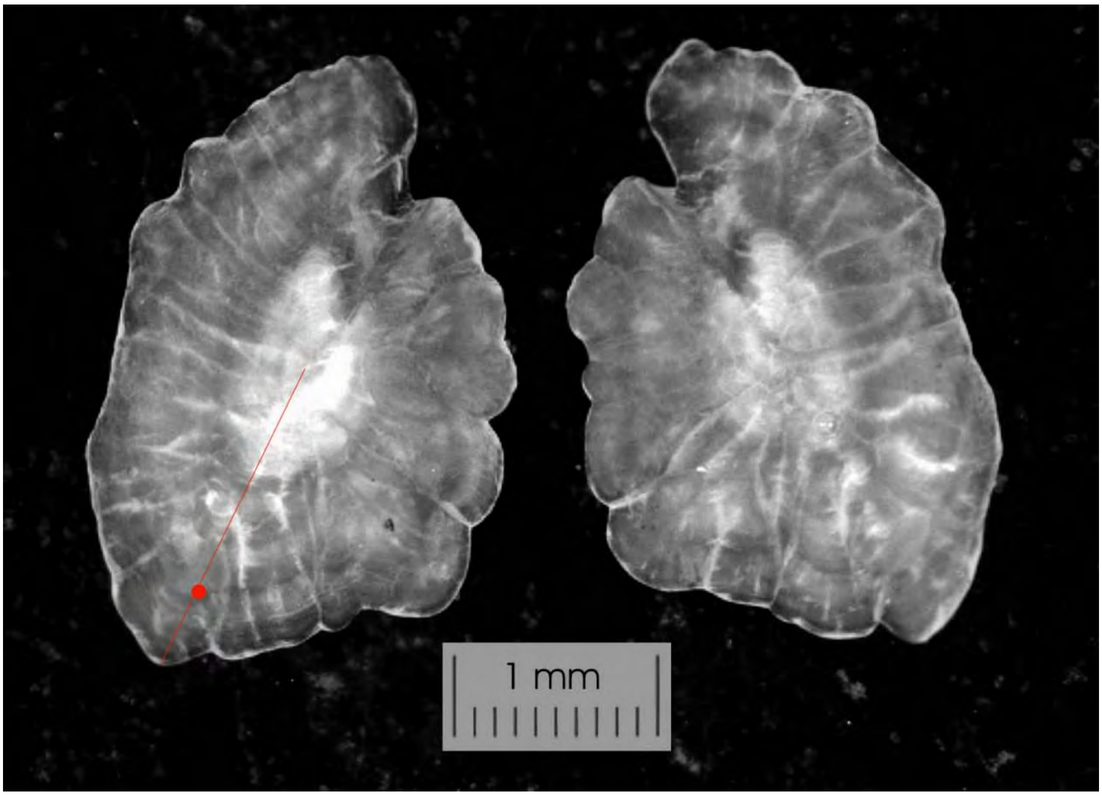

Figure 25 : Image of *M. barbatus* (age : 1 ; length : 155mm , Sex : M , month of capture : 11).

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The list of the readers in the workshop with the expertise level by stock is presented in the following table.

Country	Participant in workshop (preliminary list)	Age reader	Expertise level on M. surmuletus otoltiths	Expertise level on M. barbatus otoltiths	Stock	Stock assessment
France	Kélig Mahé	N				
	Romain Elleboode	Y	high	intermediate	IV, VIId, GSA 7	N
Greece	Chryssi Mytilineou	N				
	Katerina Anastasopoulou	Y	high	intermediate	GSA 20, 22	N
Italy	Pierluigi Carbonara	Y	high	high	GSA 10	N
	Alessandro Ligas	Y	high	high	GSA 9	N
Cyprus	Charis Charilaou	Y	intermediate	intermediate	GSA 25	N
	Antonis Petrou	Y	intermediate	intermediate	GSA 25	N
Spain	Antoni Quetglas	Y	intermediate	intermediate	GSA 05	N
	Francesc Ordines	Y	intermediate	intermediate	GSA 05	N

The list of the participants in the otoliths exchange is presented in the following table.

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Annex 2: Agenda

Monday 30/03/09

16:00–17:30: introduction, logistics, time schedule and assigning responsibilities.
17:30–18:30: visit of sclerochronology laboratory

Tuesday 31/03/09

09:00–10:30: ToR a
Review information on age determination, and validation work on these species
10:30–10:45: coffee break
10:45–12:00: Exchange results by species and by techniques (Striped/Red mullet)
12:00–13:30: lunch
13:30–14:30: ToR b
Compare different otolith-based age determination methods
14:30–16:00: Evaluation of the age reading exercise
Resolve interpretation differences between readers and laboratories by performing an analysis of metric measurements of otolith structures as a solution to minimize the divergence in age estimation of Red mullet
16:00–16:15: coffee break
16:15–17:45: Evaluation of the age reading exercise
Resolve interpretation differences between readers and laboratories by performing an analysis of metric measurements of otolith structures as a solution to minimize the divergence in age estimation of Striped mullet
17:45–18:30: TNPC software presentation

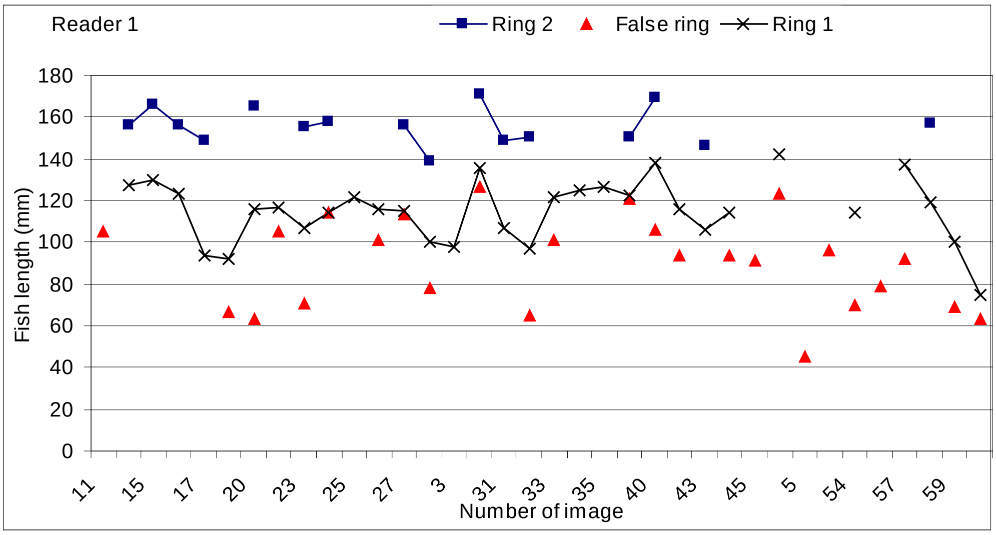
Wednesday 01/04/09

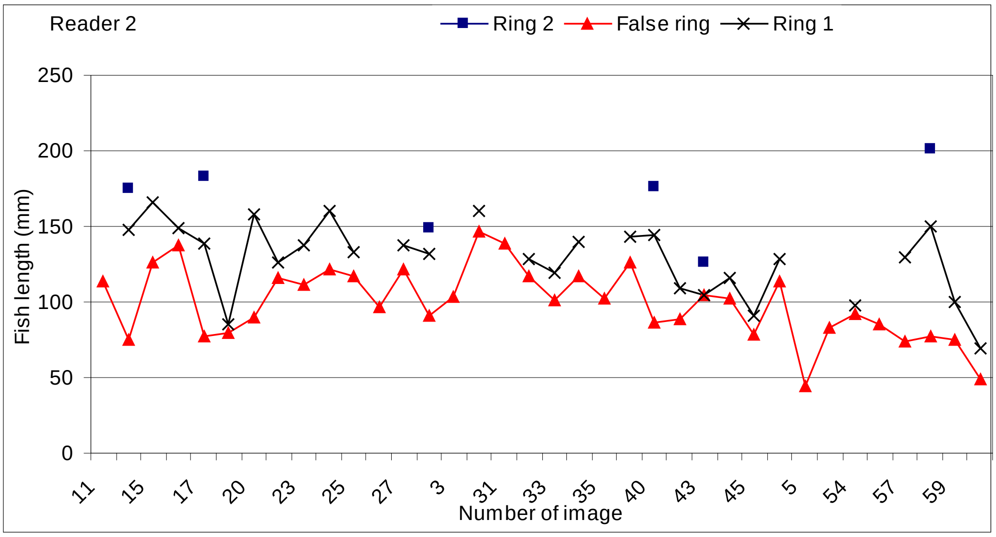
09:00–10:30: Reread of exchange otoliths (Striped mullet)
10:30–10:45: coffee break
10:45–12:00: Reread of exchange otoliths-Analysis of results (Striped mullet)
12:00–13:30: lunch
13:30–16:15: Reread of exchange otoliths-Analysis of results (Red mullet)
16:15–16:30: coffee break
16:30–18:30: ToR c
Identify sources of age determination error in terms of bias and precision, and agree on a common ageing criteria

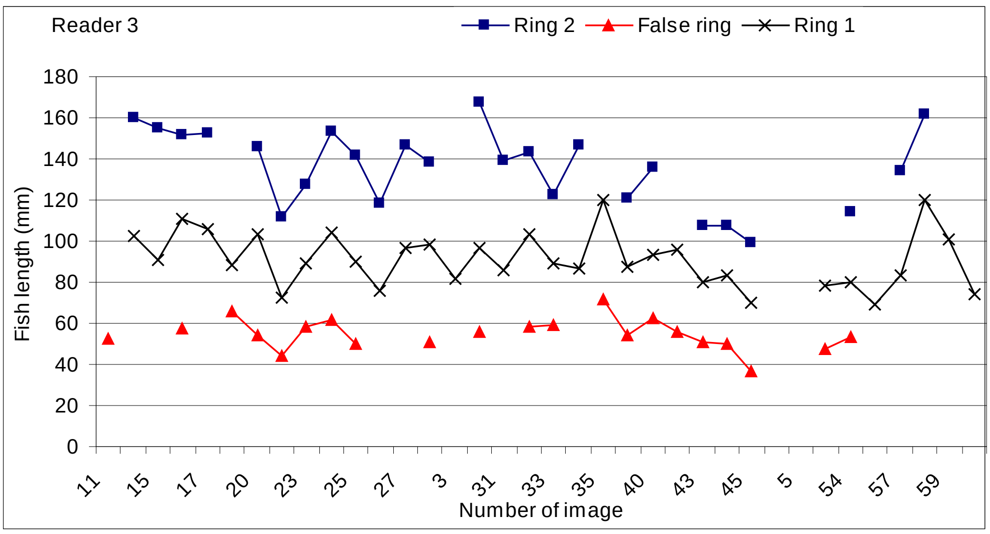
Thursday 02/04/09

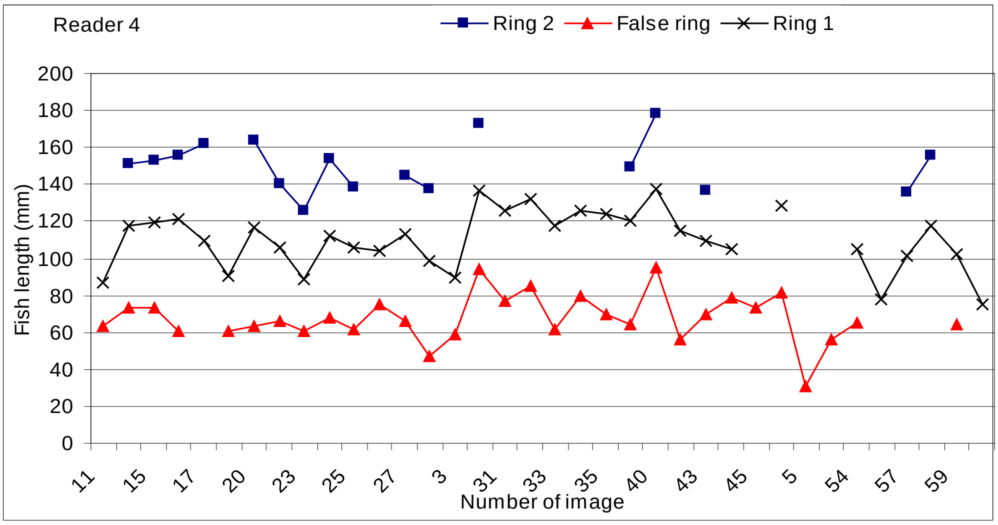
09:00–10:30: ToR e
Reference collection of otoliths
10:30–10:45: coffee break
10:45–12.00: ToR d
Guidelines for the interpretation of growth structures in otoliths
12:00–13:30: lunch
13:30–16:00: Draft report, Recommendation
16:00–16:15: coffee break
16:15–18:30: Plenary final
Draft reports, Recommendations, Agreements

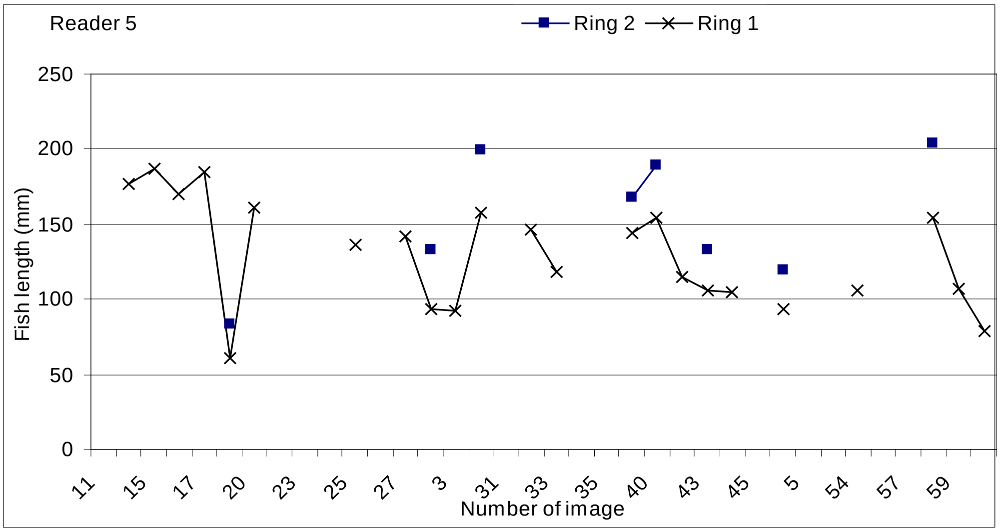
Annex 3: Back-calculations of the fish length per each reader for the *M. barbatus*

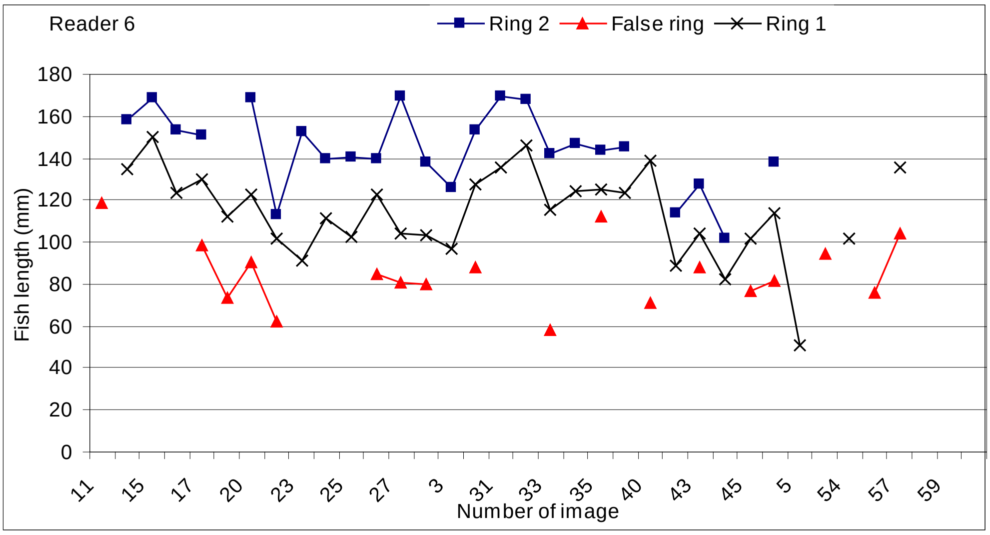




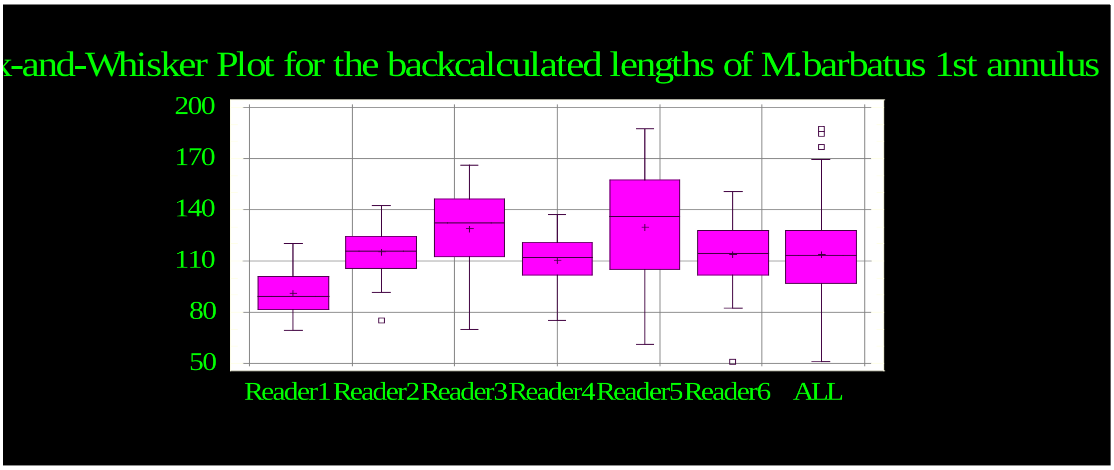


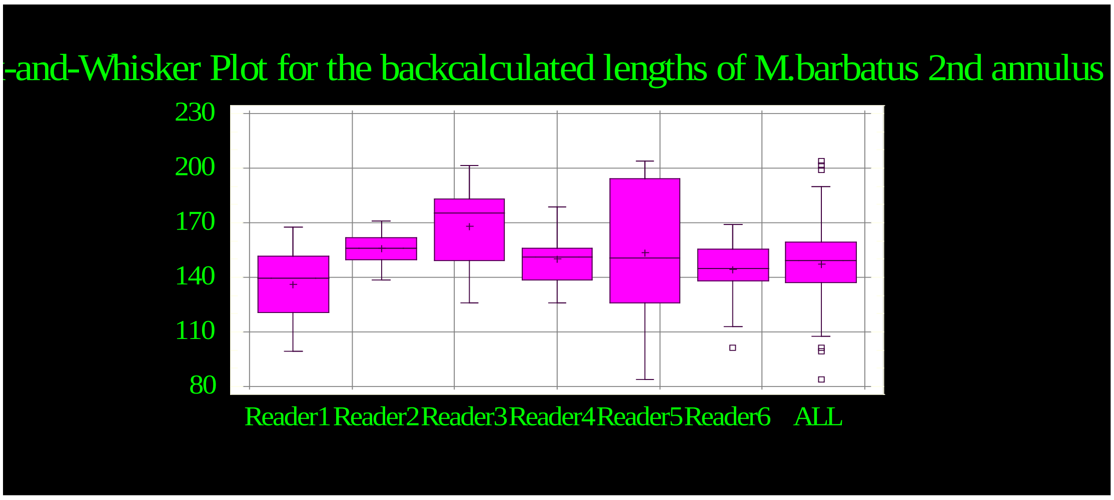

Reader 4
Ring 2
False ring
Ring 1
Fish length (mm)
0
20
40
60
80
100
120
140
160
180
200
11
15
17
20
23
25
27
3
31
33
35
40
43
45
5
54
57
59
Number of image


Reader 5
Ring 2
Ring 1
Fish length (mm)
0
50
100
150
200
250
11
15
17
20
23
25
27
3
31
33
35
40
43
45
5
54
57
59
Number of image

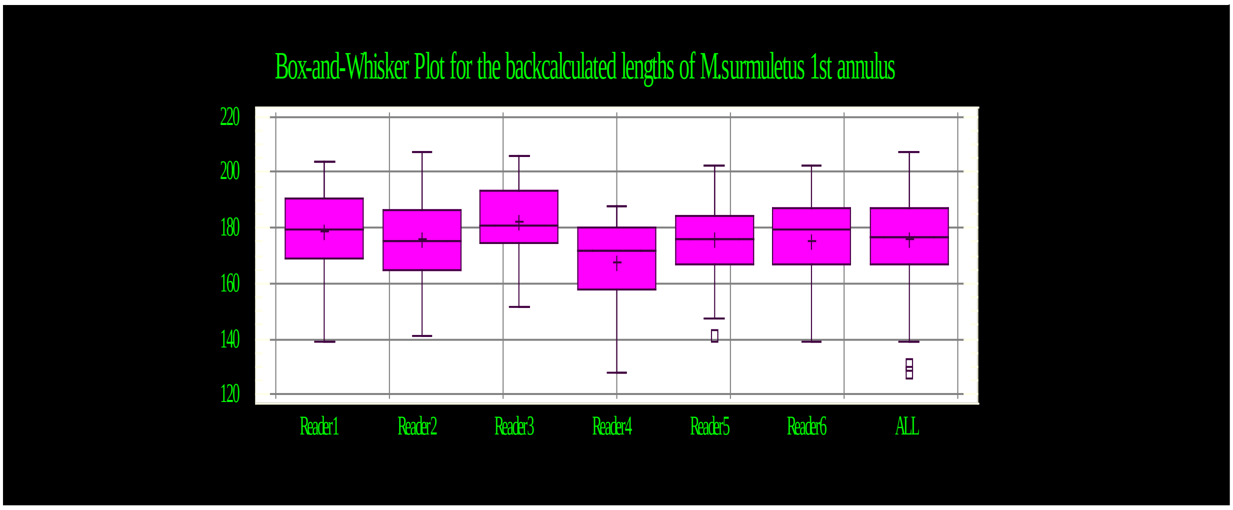

Reader 6
Ring 2
False ring
Ring 1
Fish length (mm)
0
20
40
60
80
100
120
140
160
180
11
15
17
20
23
25
27
3
31
33
35
40
43
45
5
54
57
59
Number of image

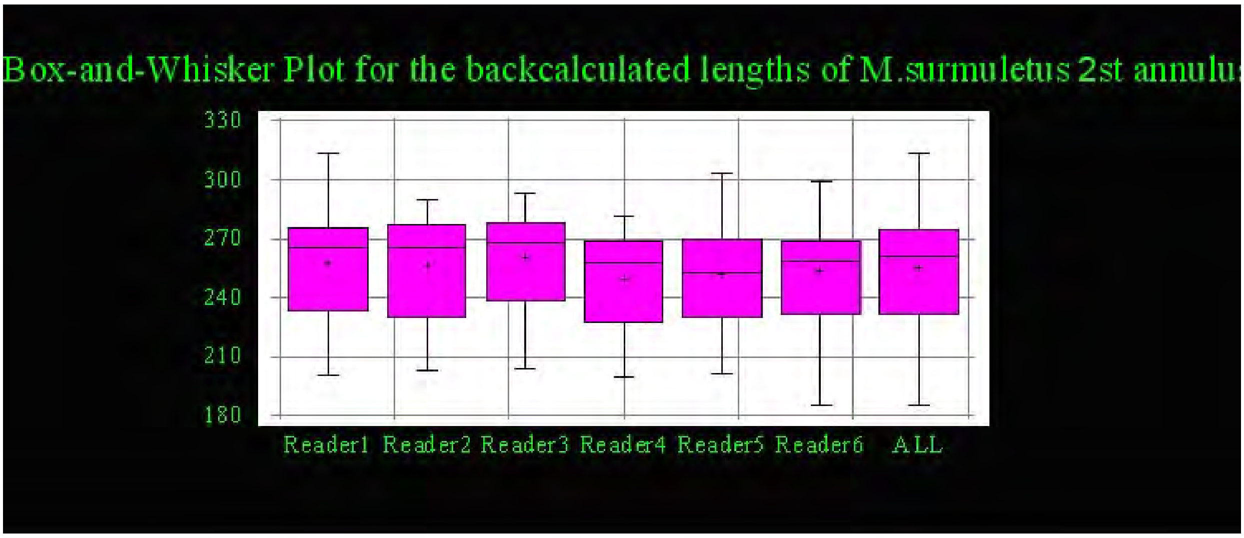
Annex 4: Box and Whiskers plots per reader for the backcalculated lengths of the 1st and 2nd annulus of *M. barbatus*.





Annex 5: Box and Whiskers plots per reader for the backcalculated lengths of the 1st and 2nd annulus of *M. surmuletus*.





Annex 6: WKACM terms of reference for the next meeting

A **Workshop on Age reading of Red mullet *Mullus barbatus* and Striped mullet *Mullus surmuletus*** [WKACM] (Chair: xxxx) will be held by xxxx from xxxx 2011 to:

a) Review the results of the new exchanges and compare with those of the previous workshop

b) Clarify the interpretation of annual rings and use various validation methods

c) Improve the protocol of the guidelines

d) Create a reference collection of well defined otoliths

e) Improve the recommendations

WKACM will report by 2010 for the exchange and by 2011 for the workshop to the attention of the PGCCDBS and ACOM.

Supporting Information

Priority:	Age determination is an essential feature in fish stock assessment to estimate the rates of moralities and growth. In order to arrive at appropriate management advice ageing procedures must be reliable. Otolith processing methods and age reading methods might differ considerably between countries. Therefore, otolith exchanges should be carried out on a regular basis, and if serious problems exist age reading workshops should be organised to solve these problems.
Scientific justification and relation to action plan:	The aim of the workshop is to identify the present problems in Mullus spp. age determination, improve the accuracy and precision of age determinations and spread information of the methods and procedures used in different ageing laboratories. Term of Reference a) Evaluation of the exchange experiments results Term of Reference b) Clarify the interpretation and improve the validation of annuli Term of Reference c) Improve the protocol of the guidelines on the age reading Term of Reference d) Create reference collections of well defined otoliths Term of Reference e) Improve the recommendations
Resource requirements:	
Participants:	In view of its relevance to the DCR, the Workshop is expected to attract wide interest from both Mediterranean EU and ICES Member States.
Secretariat facilities:	
Financial:	Additional funding will be required for facilitate the attendance of the scientists. The workshop will be eligible under the E.U. - DCR.
Linkages to advisory committees:	ACOM
Linkages to other committees or groups:	WGNEW
Linkages to other organizations:	There is a direct link with the EU DCR and outcomes from this Workshop will be of interest to several RFOs

Annex 7: Recommendations

We suggest that each Expert Group collate and list their recommendations (if any) in a separate annex to the report. It has not always been clear to whom recommendations are addressed. Most often, we have seen that recommendations are addressed to:

- Another Expert Group under the Advisory or the Science Programme;
- The ICES Data Centre;
- Generally addressed to ICES;
- One or more members of the Expert Group itself.

Recommendation	For follow up by:
1. The precision and the agreement of age determination in both Mullus species but particularly in M. barbatus need be improved	PGCCDBS, PGMED, ICES, WGNEW
2. New exchange with new sets of M. surmuletus otoliths from Mediterranean, the Biscay Gulf and the English Channel should be organised in order to detect differencies between areas	PGCCDBS, PGMED, ICES, WGNEW
3. New exchange for a new set of M. barbatus otoliths from the Mediterranean should be examined in order to clarify the ageing in this species.	PGCCDBS, PGMED, ICES, WGNEW
4. After establishing an agreement for the reading guidelines, a protocol needs to be developed which will be improved by the time	PGCCDBS, PGMED, ICES, WGNEW
5. More validation studies (e.g. daily increament studies, tagging, length based analysis, marginal increamrnt analysis etc.) are necessary for both species	PGCCDBS, PGMED, ICES, WGNEW
6. A new Workshop is necessary to be organised in 2011 to take into account the results of the new exchanges. The terms of reference could be the following: Evaluation of the exchange experiments results Clarify the interpretation and improve the validation of annuli Improve the protocol of the guidelines on the age reading Create reference collections of well defined otoliths Improve the recommendations	PGCCDBS, PGMED, ICES, WGNEW

After submission of the report, the ICES Secretariat will follow up on the recommendations, which will also include communication of proposed terms of reference to other ICES Expert Group Chairs. The "Action" column is optional, but in some cases, it would be helpful for ICES if you would specify to whom the recommendation is addressed